

human vaccines. After several passages and plaque purifications, two Vero-adapted high growth influenza H5N1 vaccine viruses (Vero-15 and Vero-16) have been selected and could reach high virus titer ( $10^8$  TCID<sub>50</sub>/ml) in Vero cells in T flasks. After tested with NIBRG-14 standard antiserum provided by the NIBSC, antigenicity of the Vero-15 and Vero-16 viruses remain similar to the NIBRG-14 virus. In addition, the Vero-15 and Vero-16 viruses do not have any nucleotide difference in HA and NA gene segments compared with the NIBRG-14. For process development, Vero cells were further cultured on cytodex 1 microcarriers in spinner flasks. Vero cells grew to  $3 \times 10^6$  cells/ml with a seeding density of  $4.4 \times 10^5$  cells/ml in 5 g/L microcarriers and peak virus titers reached  $10^9$  TCID<sub>50</sub>/ml. In conclusion, the Vero-15 and Vero-16 viruses are suitable for production of influenza H5N1 vaccines in Vero cells. Their other six internal gene segments could also be used to generate vaccine seed viruses of other influenza subtypes for production in Vero cells.

doi:10.1016/j.ijid.2008.05.687

43.008

#### Induction of IFN- $\gamma$ and IL-17 by Pneumococcal Surface Protein C-Based Vaccines does not Protect Against a *Streptococcus pneumoniae* Invasive Challenge

D.M. Ferreira, M. Darrieux, D.A. Silva, L.C.C. Leite, P.L. Ho, E.N. Miyaji, M.L.S. Oliveira\*

Centro de Biotecnologia, Instituto Butantan, São Paulo, Brazil

*Streptococcus pneumoniae* (pneumococcus) is an important pathogen that causes pneumonia, meningitis and otitis media. Invasive diseases usually follow colonization of the respiratory tract and may be favored by factors that cause immunosuppression. Several pneumococcal proteins actively participate of these events playing different roles such as facilitating bacterial adhesion to epithelial cells or evasion from the immune system. Pneumococcal Surface protein C (PspC) is a virulence factor that has been implicated both in colonization and in invasive phases of pneumococcal diseases. Antigen delivery systems based on live recombinant lactic acid bacteria represents a promising strategy for mucosal vaccination, since they are able to elicit both systemic and mucosal immune responses. In the present work, we have evaluated the immune response and the protective activity of nasal vaccines composed of recombinant PspC (rPspC) or *Lactobacillus casei* expressing PspC (L.c.PspC). Nasal immunization of mice with both formulations did not elicit the production of anti-PspC IgG or IgA. On the other hand, ELISPOT and cytokine ELISA analysis of cultures obtained from mice 13 h after intranasal challenge with a virulent pneumococcal strain, showed an increase in IFN- $\gamma$  secretion in lung cells from mice immunized with L.c.PspC and to a lesser extent, rPspC. This cytokine was also produced by spleen cells from mice immunized with both formulations. Production of IL-17 by lung cells was observed in the group immunized with rPspC whereas immunization with L.c.PspC induced the production of this cytokine only by spleen cells. IL-17 has already been implicated in

cytokine or IFN- $\gamma$  by our vaccines did not confer protection against an invasive challenge with pneumococci. Further studies will be necessary for the evaluation of protection against nasal colonization.

*Financial Support:* FAPESP, Fundação Butantan, Millenium Institute-Gene Therapy Network (MCT-CNPq).

doi:10.1016/j.ijid.2008.05.688

43.009

#### DNA Vaccine Construct in the Presence of EV71 IRES Elicited Higher Neutralizing Antibody Titre

N.A. MatRahim\*, S. AbuBakar

University Malaya, Kuala Lumpur, Malaysia

Since 1997, large epidemics of EV71 infection have been reported in East and Southeast Asia. The virus has caused numbers of outbreak and infection associated with fatal neurological complications, however no vaccine nor antiviral against EV71 are available. In our study, we have developed DNA-based vaccines against EV71. The vaccines consist of structural protein VP1 of human enterovirus 71 (EV71) as fusion proteins with enhanced green fluorescent protein (EGFP), with and without the presence of internal ribosome entry site (IRES) at the 5'-end; IRESVP1/EGFP and VP1/EGFP. Expressions of both constructs were evaluated in vitro using Vero and SK-N-MC cells, and later in vivo in murine model. Evaluation of in vitro expression showed that the VP1 gene expressed by 5'UTR-VP1/EGFP is higher in comparison to construct without IRES; VP1/EGFP, in both Vero and SK-N-MC cells. The ability of the constructed DNA vaccines in eliciting immune responses were evaluated in vivo using murine model. The mice were immunized with 2 dosages of DNA vaccine followed by experimental challenge. In vivo evaluation showed that the mice group immunized with IRESVP1/EGFP confer a higher neutralizing antibody titer in comparison to the VP1/EGFP. Results from our study not only demonstrate the potential of VP1-based DNA vaccine but also suggests the feasibility of using IRES to generate better protective immunity in mice against EV71 and the possibility to develop safe vaccine against enterovirus infection.

doi:10.1016/j.ijid.2008.05.689

43.010

#### Protective Immunity Induced by Baculovirus-Expressed Rabies Glycoprotein and Recombinant Adenovirus Expressing Its Protein

K.K. Lee\*, B.J. So, C.K. Park, J.K. Oem, S.H. Kim, H.R. Kim, C.H. Kwon, Y.S. Joo

National Veterinary Research and Quarantine Service, Anyang, Republic of Korea

*Background:* Since rabies virus (RV) infection is fatal for both human and animals, the protective immunization by vaccines is of critical importance for disease control and prevention. Several recombinant protein and live viruses of rabies have been constructed and tested for their